bio-protocol Preprint

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Colony formation assay

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An abbreviated version of this protocol was published in eLIFE in Mar 2019

Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury

DOI: 10.7554/eLife.43882

Detailed protocol

Dear May,
Thank you for your appreciation and interest.
As indicated in the paper, we only work on stromal cells/ cardiac fibroblasts (for colony assay) and did not sort immune cells or perform any in vitro assays with immune cells.

Please find protocol for colony assay on cardiac stromal cells here:

Non-cardiomyocyte, mononuclear cells (TIP cells) were isolated from dissected hearts from adult mice (8-12 weeks old) via mincing of tissue and digesting it in Collagenase type II (5 ml of 165 units/ml in PBS) (Worthington) in PBS at 37°C for 30 min, and having it be triturated mechanically at 10 min intervals and with myocyte/debris removed with a 40 µm filter.

- Isolation of heart
- Mincing of tissue in collagenase (5 ml)
- Incubation on rotator for 10 minutes at 37 degree Celsius
- Take supernatant and filter through 40 micron sieve
- Add 5 ml collagenase and repeat the procedure for 2 times (Total 15 ml/heart)
- Spin down at 300g for 5 min
- Resuspend in red cell lysis buffer and 1x wash with FACS buffer (2%FBS in PBS). Proceed to dead cell removal

Dead cells were removed using MACS Dead Cell Removal kit (Miltenyibiotec) before incubation with fluorophore-conjugated antibodies (listed in the paper) and sorting using FACS Aria (Becton and Dickinson) cytometer.

FACS sorted primary cells were seeded at a clonogenic density of 50 cells/cm^2 (500 cells per well of 6-well plate) and were cultured in α-Minimal Essential Medium (α-MEM) containing 20% FBS+1% Pen/Strep at 37°C in a humidified 2% O_2 and 5% CO_2 incubator, with medium changes every 2–3 days.

After 8-day culture, colonies were rinsed with phosphate-buffered saline (PBS), fixed with 2% paraformaldehyde (PFA) and stained with 0.05% (v/v) crystal violet dye in water. Differences in colony number and size were evaluated by a two-tailed one-sample *t*-test to test for variability between individual samples.

Colony criteria:

- At least 50 cells at day 8 in hypoxia (P0)
- Small, spindle-shaped morphology cells with bipolar or multipolar prominences extending from the cell body. Colonies become highly compact with sharp edges and composed of small cell body and tight bundling.

Related files

Colony formation assay.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Patrick, R. (2020). Colony formation assay. Bio-protocol Preprint. bio-protocol.org/prep229.
2. Farbehi, N., Patrick, R., Dorison, A., Xaymardan, M., Janbandhu, V., Wystub-Lis, K., Ho, J. W., Nordon, R. E. and Harvey, R. P.(2019). Single-cell

expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. eLIFE. DOI: 10.7554/eLife.43882